with different (from 0 to 6) combinations of truncated and full-length sequences. Formation of concatemers was assessed by the use of single-molecule photobleaching and protein cross-linking. Whole-cell recordings from concatenated PANX1 constructs suggest that at least four intact C-termini are required to inhibit channel activity. In addition, as the number of intact C-termini increased, there was a progressive decrease in single channel conductance, suggesting that individual C-termini may act within the multimeric channel to inhibit channel conductance. These results provide further mechanistic insights into the regulation of PANX1 channels by the C-terminal auto-inhibitory domains.

3251-Pos Board B406
Structural Basis of Pannexin Activation
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Pannexin 1 (Panx1) is a member of a family of large-pore ion channels distantly related to invertebrate gap junction channels, the innexins. Activation of Panx1 occurs under a variety of physiological processes, but the molecular mechanism of such activation has not in general been clearly established. Recently it was shown that Panx1 could be activated by direct cleavage near the c-terminus by caspase-3. This occurs during apoptosis and leads to the release of large intracellular molecules such as ATP, which act as ''find-me'' signals enabling nearby phagocytes to quickly clear dying cells. The C-terminal domain peptide seems to function as a tethered pore blocker and is thought to have a specific interaction with structural elements within the pore to inhibit the channel. However, the basis for this structural interaction is poorly understood. Here we dissect those interactions using progressive alanine substitutions and long alanine repeats within the region distal to the caspase cleavage site. Our results suggest that any specific interaction between the pore and the C-terminus is minimal since replacement of the amino acid residues in the region adjacent to the caspase cleavage site with alanines does not produce a constitutively open channel. The most important feature of the C-terminal inhibitory peptide appears instead to simply be its length. We therefore propose that the c-terminal peptides loop back into the pore to reach a constricted region, thereby blocking the pore by sterically interfering with ion permeation. Peptides not long enough to reach this constriction within the pore are consequently unable to block the channel while longer peptides are able to block the pore in a promiscuous fashion.

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Pseudomonas Aerginosa Outer Membrane Carboxylate Channels Examined at the Single-Molecule Level Reveals Conserved Selectivity within each Subfamily
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Pseudomonas aerginosa is a Gram-negative bacterium, which employs unique outer membrane proteins for the uptake of small, water-soluble molecules. Here, we explore the two subfamilies of the outer membrane carboxylate channels, OccK and OccD, using single-molecule electrophysiology to probe the specific signature of each of the members of these two families. The seven member OccK family displays a broad range of unitary conductance values which includes low (~40−100 pS) and medium (~100−380 pS) conductance. These values are broader than what was expected from structural studies alone. It was also found that the OccK subfamily displays conserved anion selectivity, coinciding with the location of a net pool of positive charges within the channels' constriction sites. Single-channel activities of 6 members of the OccD subfamily also displayed a broad range of unitary conductance values between 20 and 670 pS. Single-channel electrophysiology studies indicated that the OccD subfamily members are all cation selective. Together these findings lead to a better understanding of the diversity within the outer membrane carboxylate channels of *Pseudomonas aerginosa*.

3253-Pos Board B408
A Simulation Approach to Molecular Transport in Bacterial Reaction Chambers
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Bacterial microcompartments (BMCs) are capsid-like assemblies that serve as simple protein-based metabolic organelles in bacterial cells. BMCs consist of a few thousand shell proteins that encapsulate specific enzymes, thus sequestering and/or enhancing certain metabolic pathways that often involve toxic intermediates. BMCs represent promising targets for rational modification in bionanotechnology. Crystallographic structures of some BMC proteins suggest that dynamic pores may be present within shell assemblies, providing the means for the controlled transport of substrates, products, and cofactors in the absence of a selectively permeable membrane. However, experimental evidence regarding BMC molecular transport remains inconclusive. In this study, all-atom, explicitly solvated molecular dynamics simulations have been used to generate microsecond-timescale sampling of molecular transport through several homohexameric shell assemblies from the archetypal BMC, the carboxysome. The carboxysome is specialized for carbon fixation in cyanobacterial cells, converting atmospheric carbon dioxide into organic compounds via the encapsulated enzyme ribulose-1,5-bisphosphate carboxylase oxygenase (RuBisCO). It has been hypothesized that the shell may be porous to bicarbonate which is sequestered within the carboxysome following conversion to carbon dioxide, whilst oxygen may be excluded as it is known to inhibit the fixation process by RuBisCO. Molecular simulations have been used to characterize the permeability properties of carboxysome oligomers to water, salt, oxygen, carbon dioxide, and bicarbonate. Probability maps and corresponding potentials of mean force for selective transport of metabolic intermediates have been derived, and related to the size, electrostatics, and hydrogen-bonding characteristics of carboxysome capsid subunits. Moreover, conformationally dynamic regions of the BMC subunits were observed to partially occlude the pores, suggesting the possibility of gated transport. Overall, our observations provide insight into the molecular mechanisms of microcompartment transport, with fundamental implications for biological protein assemblies.

3254-Pos Board B409
An Unusual Aquaporin-Like Metalloid Boric Acid Channel in *Arabidopsis*
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The Major Instrinsic Protein/aquaporin superfamily is an ancient family with a conserved protein fold that forms a channel that mediates the bidirectional transport of water and uncharged solutes. The evolution of land plants was accompanied by a major diversification of the MIP gene family that resulted in the acquisition of new transport functions. Among these divergent plant specific MIP subfamilies are the ''nodulin 26 intrinsic proteins'' (NIPs), based on structural and functional homology to soybean nodulin 26. NIPs are divided into three subfamilies (NIP I, II and III) which show low to absent water permeability but have acquired the ability to transport a variety of uncharged solutes ranging from glycerol to boric acid to protonated lactic acid. In the present work we characterize the transport and gating properties of an unusual NIP II protein from *Arabidopsis* pollen microspores, AtNIP7;1 which is related to the boric acid channels AtNIP6;1 and 5;1, necessary for the uptake of this critical micronutrient. Functional characterization of NIP7;1 shows that unlike NIP5;1 and NIP6;1 which form constitutive boric acid channels, the intrinsic boric acid transport activity of NIP7;1 is extremely low. Molecular modeling suggests that a conserved tyrosine residue (Tyr 81) located in the transport pore stabilizes a closed conformation of the pore. Molecular dynamics simulation suggests that the closed conformation is stabilized by hydrogen bonding between the Tyr81 hydroxyl group and Arg 220 of the canonical ''aromatic-arginine'' selectivity filter. Since boric acid is both essential nutrient as well as a toxic compound at high concentrations, it is proposed that Tyr 81 modulates transport and provides an additional level of regulation of uptake of boric acid in male gametophyte development.

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Catsper Channel Organizes and Regulates Calcium Signaling Molecules in Spermatozoa
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Ejaculated mammalian spermatozoa gain the capacity to fertilize the egg within the female reproductive tract. Called capacitation, these changes to sperm include alterations in motility, membrane composition, and widespread protein tyrosine phosphorylation. Hyperactivated motility, the amplified, asymmetric movement of the tail, requires the sperm tail-specific Ca^{2+} channel, Cationic channel of sperm (CatSper). Calcium-mediated intraflagellar signaling is poorly understood, in part because flagellar diameters are less than 1 μm, preventing the resolution of specific molecular structures by conventional fluorescence microscopy. Here, we employed three-dimensional stochastic optical reconstruction microscopy (STORM) to determine how CatSper channel complex and other Ca^{2+} signaling molecules are organized. We find that the CatSper channel forms four linear nanodomains with calcium signaling molecules near the plasma membrane along the flagella. Strikingly, lack of the CatSper channel results in delocalization of Ca^{2+} signaling molecules. The spatial organization of protein components in CatSper signaling provides insights into Ca^{2+} signal transduction in regulation of sperm motility.